

Table 1. Amino acid sequence of peptides hydrolyzed by human glandular kallikrein 2 (HK2)

G	K	A	R	A	F	(SEQ. ID NO: 1)
G	K	A	V	R	Q	(SEQ ID NO: 2)
G	K	A	Y	F	M	(SEQ ID NO: 3)
G	K	A	E	K	V	(SEQ ID NO: 4)
G	K	A	F	R	K	(SEQ ID NO: 5)
G	K	A	K	P	R	(SEQ ID NO: 6)
G	K	A	A	Y	Y	(SEQ ID NO: 7)
G	K	A	M	Y	H	(SEQ ID NO: 8)
G	K	A	F	R	R	(SEQ ID NO: 9)
G	K	A	L	Q	R	(SEQ ID NO: 10)
G	K	A	M	R	Q	(SEQ ID NO: 11)
G	K	A	A	L	M	(SEQ ID NO: 12)
G	K	A	Q	G	F	(SEQ ID NO: 13)
G	K	A	N	M	N	(SEQ ID NO: 14)

Random library constructed with sequence

NO₂-Y-G-K-A-X₁-X₂-X₃-Dap-F-K(ABZ)

Where NO₂-Y is nitrotyrosine quencher

X₁, X₂, X₃ are random amino acids consisting

of all natural L-amino acids except cysteine (n=19)

Dap is diaminopropanoate; K(ABZ) is lysine coupled to

fluorophore-aminobenzoyl acid (ABZ)

HK2 cleavage sites denoted by single or double //

FIGURE 1

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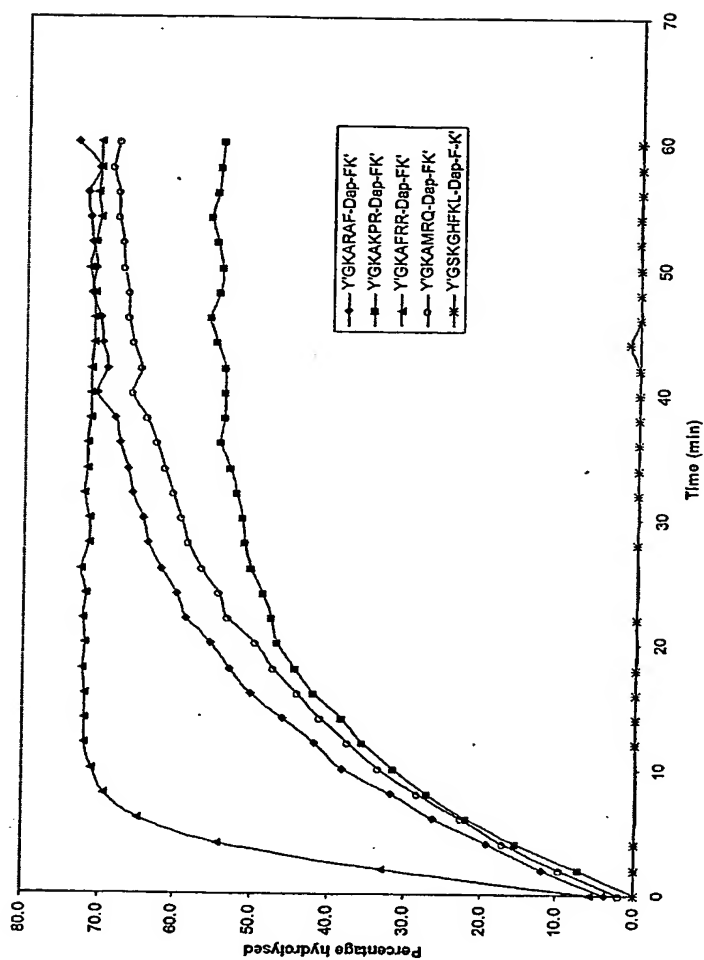


Figure 2

hK2 Hydrolysis Sites

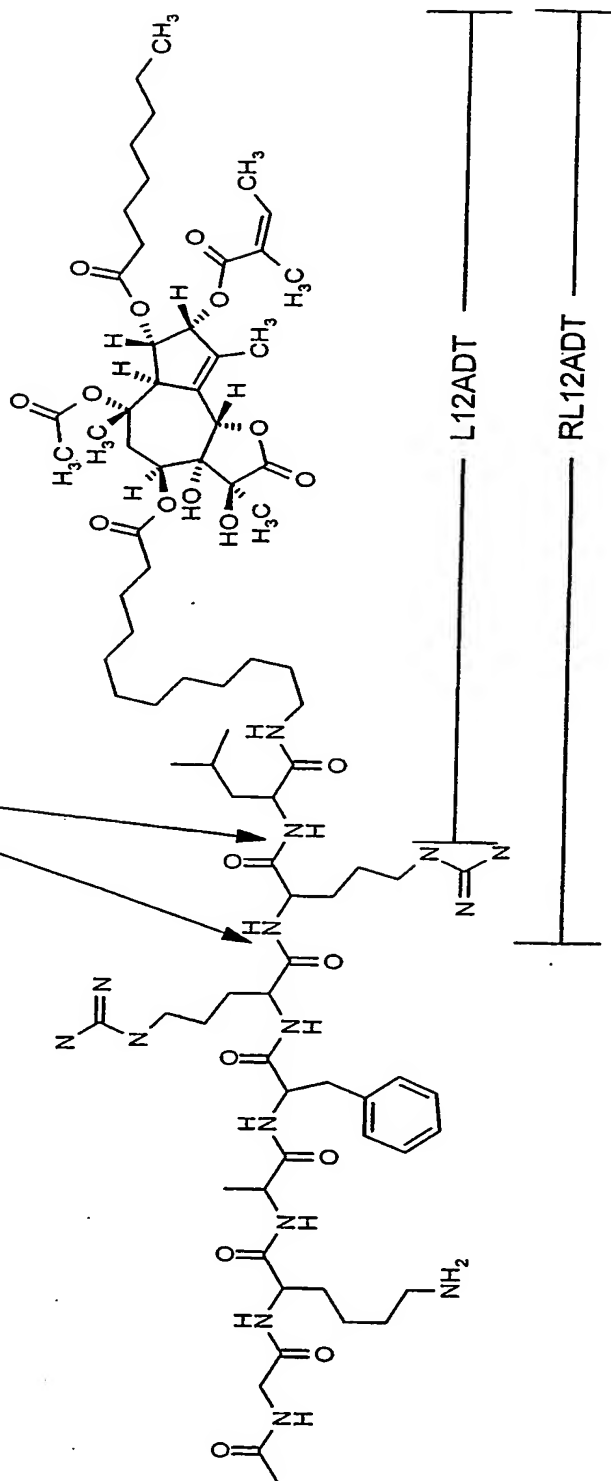
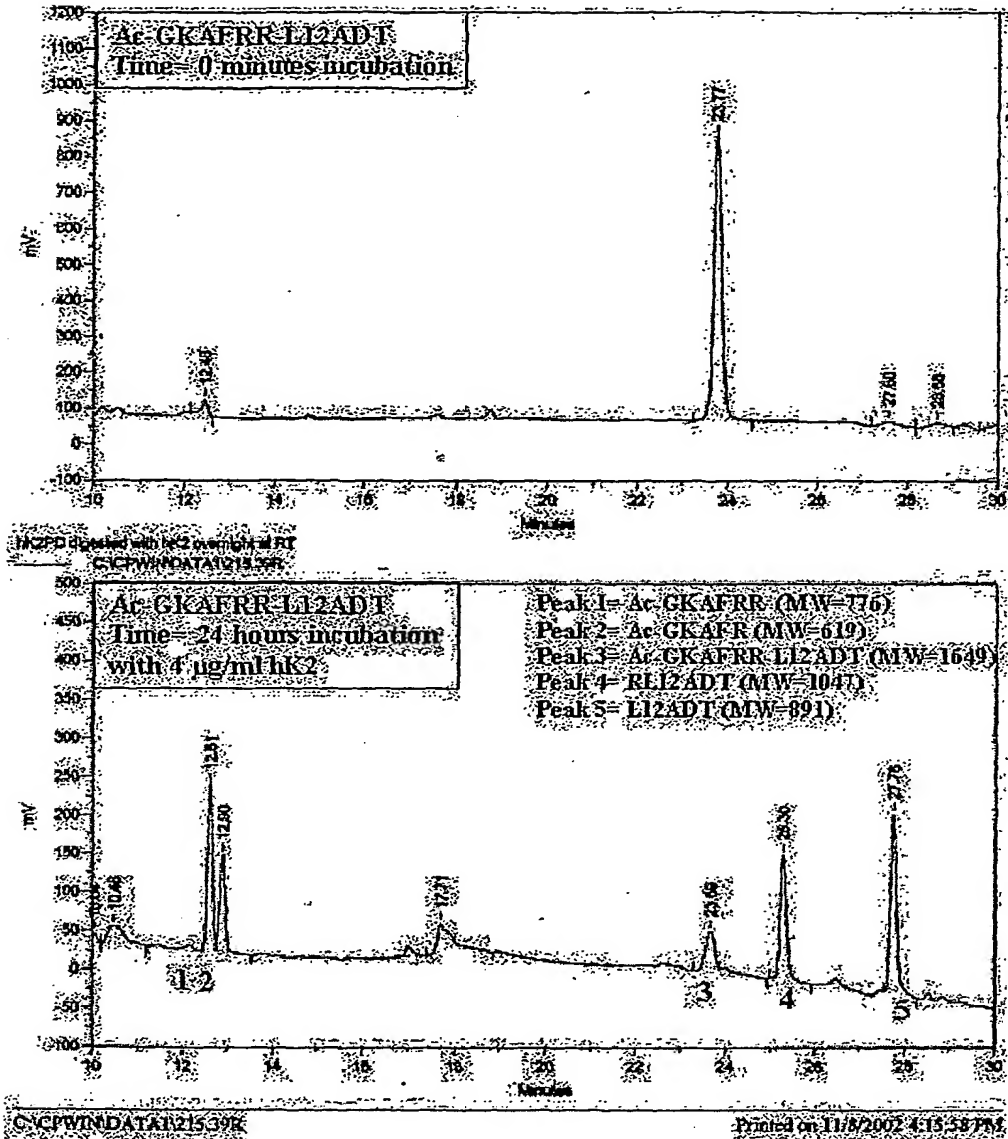
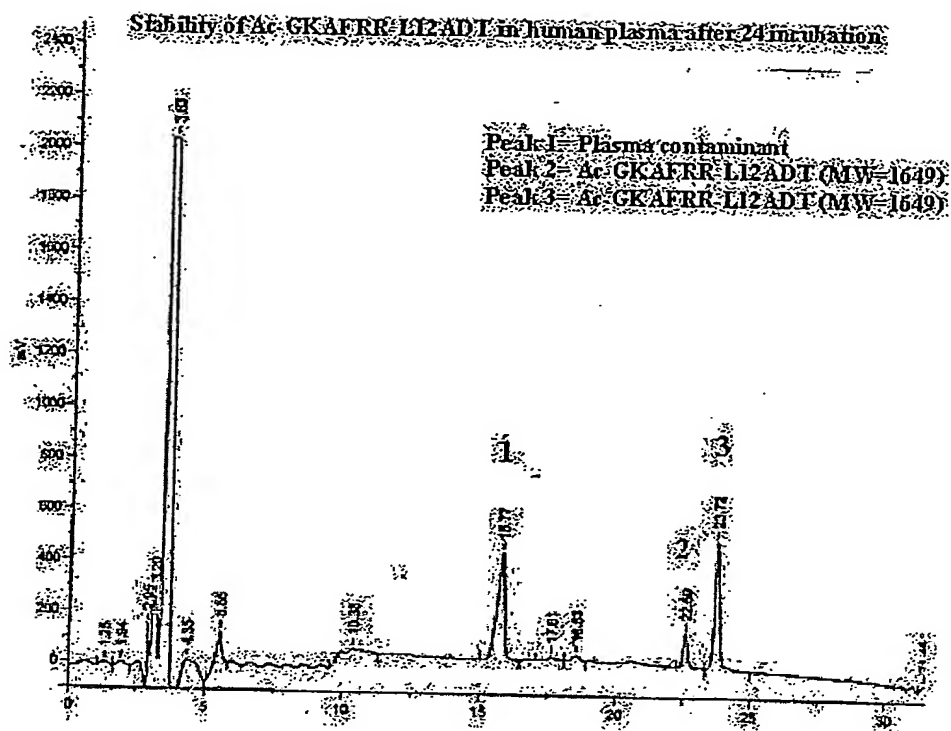


FIGURE 3



HPLC analysis of hydrolysis of hK₂ prodrug Ac-GKAFRR-LI2ADT by hK₂ (4 µg/ml) over 24 hr. incubated in 50 mM TRIS, 0.1 M NaCl, pH 7.3 at room temperature. Mass of each peak confirmed by MALDI-TOF mass spectrometric analysis (see figure 3 for mass profiles).

FIGURE 4



HPLC analysis of Ac-GKAFRR-L12ADT incubated in 50% human plasma for 24 hrs at room temperature. Peak 1 represents unidentified plasma contaminant that was also present in control plasma. Peak 2 and 3 both represent Ac-GKAFRR-L12ADT as confirmed by MALDI-TOF mass spectrometric analysis.

FIGURE 5

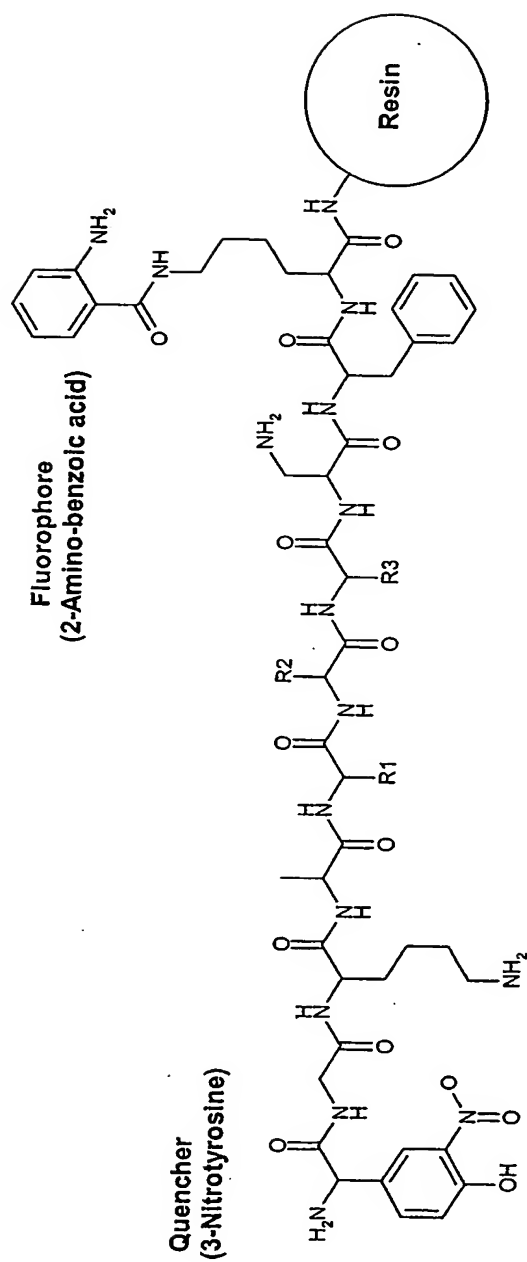


Figure 6

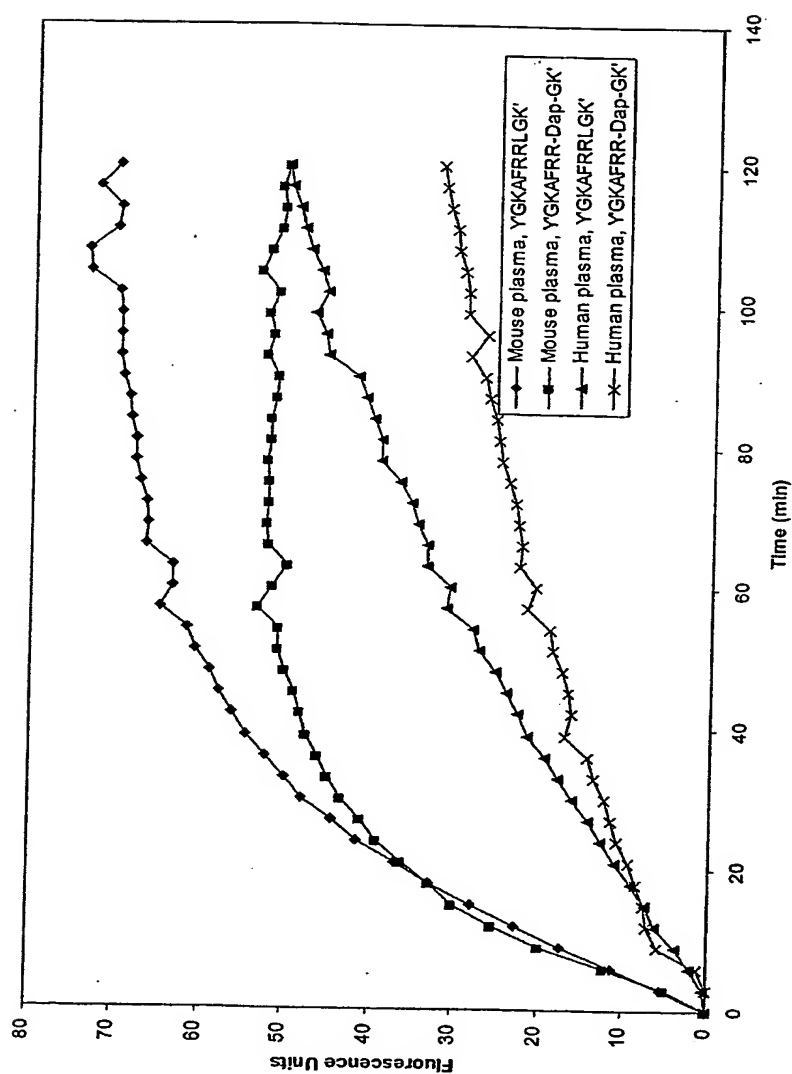


FIGURE 7

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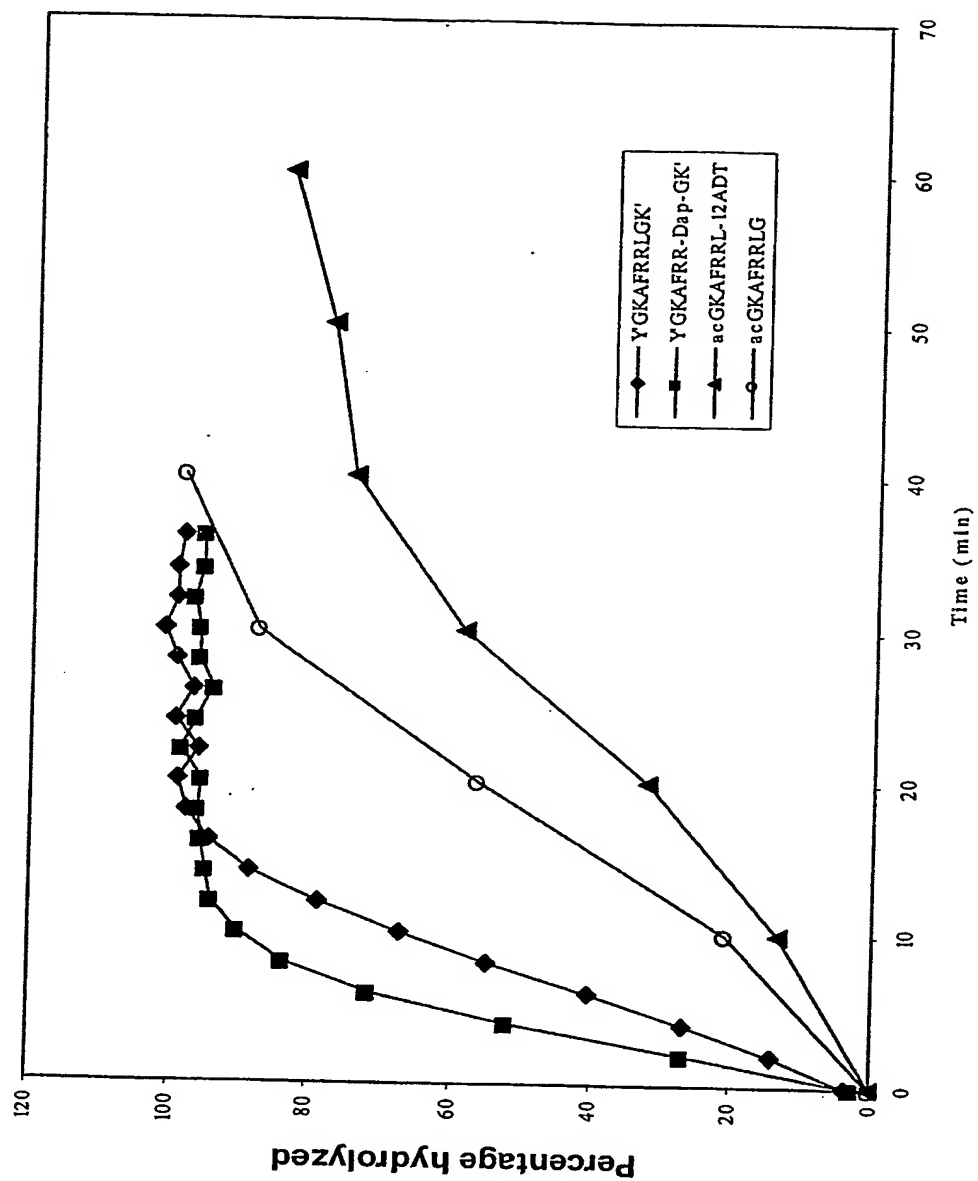


FIGURE 8

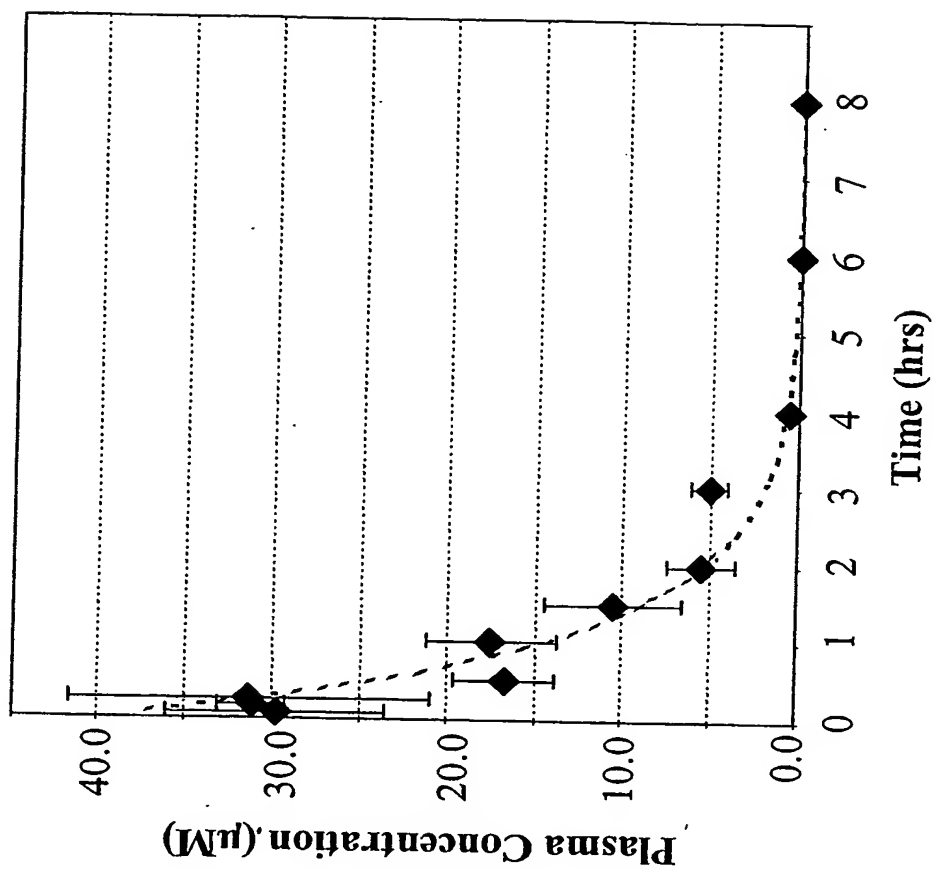


FIGURE 9